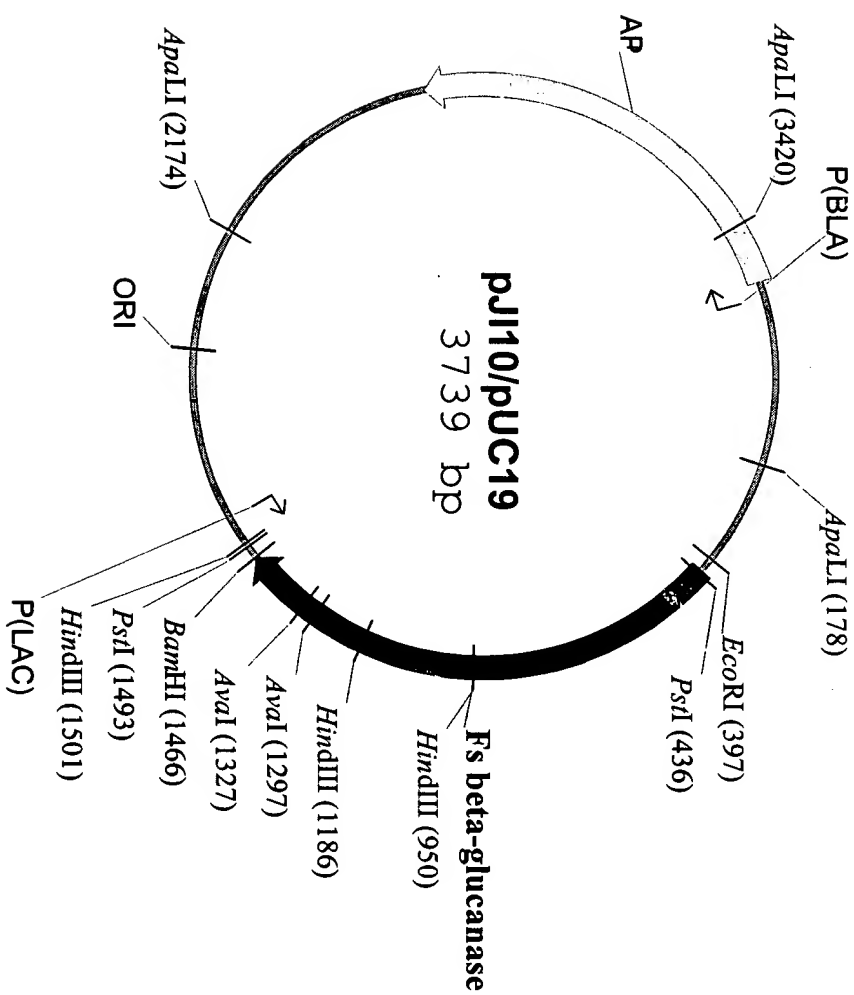


Fig. 1



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Fig. 2 The amino acid sequence in Fig. 1's SEQ ID NO: 1
 the DNA sequence in Fig. 2 is SEQ ID NO: 4

ATGGTTAGCGCAAAGGATTTTAGCGGTGCCGAACCTCTACACGTTAGAAGAAGTTCAGTAC
 M V S A K D F S G A E L Y T L E E V Q Y 20

GGTAAGTTTGAAGCCCGTATGAAGATGGCAGCCGCATCGGGAACAGTCAGTTCCATGTTC
 G K F E A R M K M A A A S G T V S S M F 40

CTCTACCAGAATGGTTCCGAAATCGCCGATGGAAGGCCCTGGGTAGAAGTGGATATTGAA
 L Y Q N G S E I A D G R P W V E V D I E 60

GTTCTCGGCAAGAATCCGGGCAGTTTCCAGTCCAACATCATTACCGGTAAGGCCGGCGCA
 V L G K N P G S F Q S N I I T G K A G A 80

CAAAAGACTAGCGAAAAGCACCATGCTGTTAGCCCCGCCGCCGATCAGGCTTTCACACC
 Q K T S E K H H A V S P A A D Q A F H T 100

TACGGTCTCGAATGGACTCCGAATTACGTCCGCTGGACTGTTGACGGTCAGGAAGTCCGC
 Y G L E W T P N Y V R W T V D G Q E V R 120

AAGACGGAAGGTGGCCAGGTTTCCAACCTTGACAGGTACACAGGGACTCCGTTTAAACCTT
 K T E G G Q V S N L T G T Q G L R F N L 140

TGGTCGTCTGAGAGTGCGGCTTGGGTGGCCAGTTCGATGAATCAAAGCTTCCGCTTTTC
 W S S E S A A W V G Q F D E S K L P L F 160

CAGTTCATCAACTGGGTCAAGGTTTATAAGTATACGCCGGGCCAGGGCGAAGGCCGCAGC
 Q F I N W V K V Y K Y T P G Q G E G G S 180

GACTTTACGCTTGACTGGACCGACAATTTTGACACGTTTGATGGCTCCCGCTGGGGCAAG
 D F T L D W T D N F D T F D G S R W G K 200

GGTGACTGGACATTTGACGGTAACCGTGTCGACCTCACCGACAAGAACATCTACTCCAGA
 G D W T F D G N R V D L T D K N I Y S R 220

GATGGCATGTTGATCCTCGCCCTCACCCGCAAAGGTCAGGAAAGCTTCAACGGCCAGGTT
 D G M L I L A L T R K G Q E S F N G Q V 240

CCGAGAGATGACGAACCTGCTCCG
 P R D D E P A P 248

Fig. 3

The amino acid sequence in Fig. 3 is SEQ ID NO: 2 ^{Fig. 3} ^{Shyur et al}

The DNA sequence in Fig. 3 is SEQ ID NO: 5

ATGGTTAGCGCAAAGGATTTTAGCGGTGCCGAAGCTCTACACGTTAGAAGAAGTTCAGTAC
M V S A K D F S G A E L Y T L E E V Q Y 20

GGTAAGTTTGAAGCCCGTATGAAGATGGCAGCCGCATCGGGAACAGTCAGTTCCATGTTT
G K F E A R M K M A A A S G T V S S M F 40

CTCTACCAGAATGGTTCCGAAATCGCCGATGGAAGGCCCTGGGTAGAAGTGGATATTGAA
L Y Q N G S E I A D G R P W V E V D I E 60

GTTCTCGGCAAGAATCCGGGCAGTTTCCAGTCCAACATCATTACCGGTAAGGCCGGCGCA
V L G K N P G S F Q S N I I T G K A G A 80

CAAAAGACTAGCGAAAAGCACCATGCTGTTAGCCCCGCCGCCGATCAGGCTTTCCACACC
Q K T S E K H H A V S P A A D Q A F H T 100

TACGGTCTCGAATGGACTCCGAATTACGTCCGCTGGACTGTTGACGGTCAGGAAGTCCGC
Y G L E W T P N Y V R W T V D G Q E V R 120

AAGACGGAAGGTGGCCAGGTTTCCAAGTTGACAGGTACACAGGGACTCCGTTTTAACCTT
K T E G G Q V S N L T G T Q G L R F N L 140

TGGTCGTCTGAGAGTGCGGCTTGGGTTGGCCAGTTCGATGAATCAAAGCTTCCGCTTTTC
W S S E S A A W V G Q F D E S K L P L F 160

CAGTTCATCAACTGGGTCAAGGTTTATAAGTATACGCCGGGCCAGGGCGAAGGCCGCAGC
Q F I N W V K V Y K Y T P G Q G E G G S 180

GACTTTACGCTTGACTGGACCGACAATTTTGACACGTTTGATGGCTCCCGCTGGGGCAAG
D F T L D W T D N F D T F D G S R W G K 200

GGTGACTGGACATTTGACGGTAACCGTGTGACCTCACCGACAAGAACATCTACTCCAGA
G D W T F D G N R V D L T D K N I Y S R 220

GATGGCATGTTGATCCTCGCCCTCACCCGCAAAGGTCAGGAAAGCTTCAACGGCCAGGTT
D G M L I L A L T R K G Q E S F N G Q V 240

CCGAGAGATGACGAACCTGCTCCGTAATTCGAGCTCCGTCGACAAGCTTGCGGCCGCACTC
P R D D E P A P N S S S V D K L A A A L 260

GAGCACCACCACCACCACCTGA
E H H H H H H *

267

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Fig. 4.

Table 1. Comparison of kinetic properties of *F. succinogenes* and *B. subtilis* 1,3-1,4- β -D-glucanases

Enzyme	Specific activity (U/mg)	k_{cat} (s^{-1})	Opt. Temperature ($^{\circ}C$)	Opt. pH
Wild-type	2065 \pm 82	1296 \pm 51	50 (at pH 6.0)	6.0-8.0
TG-Glucanase	7980 \pm 341	3695 \pm 158	50 (at pH 6.0)	6.0-8.0
PCR-TF-Glucanase	7833 \pm 334	3911 \pm 166	50 (at pH 6.0)	6.0-8.0
Lichanase (Megazyme)	118 ^a	47.2 ^a	60 (at pH 6.5) ^a	6.5-7.0 ^a
	82.6 \pm 0.96	33.0 \pm 0.38	55 (at pH 7.0)	

The kinetics was performed with lichenan (6mg/mL) as substrate in 50 mM citrate buffer (pH 6.0) or in 50mM phosphate buffer (pH 7.0), and at optimum temperature as indicated.

^a. Data was taken from *Megazyme* instruction brochure of lichenase. The kinetics was done with barley β -glucan (5mg/mL) as substrate.

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Fig. 5

Table 3. Reactivation of PCR-TF-glucanase at 25 °C after heat treatment

Heat treatment	Recovery time (min)	Relative activity (%)
90 °C, 10 min	10	68
	20	81
90 °C, 30 min	10	61
	20	67
100 °C, 10 min	10	68
	20	72
100 °C, 30 min	10	55
	20	56

Fig. 6a The no acid sequence in Fig. 6 is SEQ ID NO: 3
(part a) The DNA sequence in Fig. 6 is SEQ ID NO: 6

ATGAACATCAAGAAACTGCAGTCAAGAGCGCTCTCGCCGTAGCAGCCGCAGCAGCAGCC
M N I K K T A V K S A L A V A A A A A A 20

CTCACCACCAATGTTAGCGCAAAGGATTTTAGCGGTGCCGAACTCTACACGTTAGAAGAA
L T T N V S A K D F S G A E L Y T L E E 40

GTTCAGTACGGTAAGTTTGAAGCCCGTATGAAGATGGCAGCCGCATCGGGAACAGTCAGT
V Q Y G K F E A R M K M A A A S G T V S 60

TCCATGTTTCCTCTACCAGAATGGTTCCGAAATCGCCGATGGAAGGCCCTGGGTAGAAGTG
S M F L Y Q N G S E I A D G R P W V E V 80

GATATTGAAGTTCTCGGCAAGAATCCGGGCAGTTTCCAGTCCAACATCATTACCGGTAAG
D I E V L G K N P G S F Q S N I I T G K 100

GCCGGCGCACAAAAGACTAGCGAAAAGCACCATGCTGTTAGCCCCGCCGCCGATCAGGCT
A G A Q K T S E K H H A V S P A A D Q A 120

TTCCACACCTACGGTCTCGAATGGACTCCGAATTACGTCCGCTGGACTGTTGACGGTCAG
F H T Y G L E W T P N Y V R W T V D G Q 140

GAAGTCCGCAAGACGGAAGGTGGCCAGGTTTCCAACCTGACAGGTACACAGGGACTCCGT
E V R K T E G G Q V S N L T G T Q G L R 160

TTTAACCTTTGGTTCGTCTGAGAGTGCGGCTTGGGTTGGCCAGTTCGATGAATCAAAGCTT
F N L W S S E S A A W V G Q F D E S K L 180

CCGCTTTTCCAGTTCATCAACTGGGTCAAGGTTTATAAGTATACGCCGGGCCAGGGCGAA
P L F Q F I N W V K V Y K Y T P G Q G E 200

GGCGGCAGCGACTTTACGCTTGACTGGACCGACAATTTTGACACGTTTGATGGCTCCCGC
G G S D F T L D W T D N F D T F D G S R 220

TGGGGCAAGGGTGACTGGACATTTGACGGTAACCGTGTGACCTCACCGACAAGAATC
W G K G D W T F D G N R V D L T D K N I 240

TACTCCAGAGATGGCATGTTGATCCTCGCCCTCACCCGCAAAGGTCAGGAAAGCTTCAAC
Y S R D G M L I L A L T R K G Q E S F N 260

GGCCAGGTTCCGAGAGATGACGAACCTGCTCCGCAATCTTCTAGCAGCGCTCCGGCATCT
G Q V P R D D E P A P Q S S S S A P A S 280

TCTAGCAGTGTTCCGGCAAGCTCCTCTAGCGTCCCTGCCTCCTCGAGCAGCGCATTTGTT
S S S V P A S S S S V P A S S S S A F V 300

CCGCCGAGCTCCTCGAGCGCCACAAACGCAATCCACGGAATGCGCACAACTCCGGCAGTT

Fig. 6
(part b)

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P P S S S S A T N A I H G M R T T P A V 320
GCAAAGGAACACCGCAATCTCGTGAACGCCAAGGGTGCCAAGGTGAACCCGAATGGCCAC
A K E H R N L V N A K G A K V N P N G H 340
AAGCGTTATCGCGTGAACTTTGAACACTAA
K R Y R V N F E H * 349
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